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Double Staining Technique for Rat Foetus Skeleton in Teratological Studies

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The characteristic staining of the skeleton is an important part of the toxicological studies on the individual development. The precise and full interpretation of the data regarding the skeletal toxicity is possible only if using the "double staining method" with alizarin red and alcian blue. The Wilson section method, which is standard teratological method and still represents the most utilized technique to examine the visceral organs, was used to explore the soft tissues.

The study of teratogenicity required application of the studied substances on pregnant female Wistar rats during the organogenesis. On day 21 after conception were extracted via caesarian section and were explored for skeletal toxicity, using the method of "Double skeleton staining".

The double staining method used in the current study is a fundamental part of the teratological studies for assessment of the toxicity of the xenobiotics and non-chemical factors for the individual development.

Key words: toxicology, teratogenicity, xenobiotics, Double staining method, Wilson section method.

Introduction

The teratogenic activity of the xenobiotics is studied by means of examination for visceral and skeletal abnormalities of the fetus of laboratory animals (rats).

The characteristic staining of the skeleton is an important part of the toxicological studies on the individual development [6]. The precise and full interpretation of the data regarding the skeletal toxicity is possible only if using the "double staining method" with alizarin red and alcian blue [5, 8]. All cartilages stained in blue, and the ossified bones in red. Thus the cartilaginous and the bone part of the skeleton are examined, and any possible abnormalities could be detected. Despite the latter one requires a lot of labor and time, it is the only one that can be used to study the fetus until the pregnancy is not accomplished (20^{th} day of the pregnancy) [2, 3, 10].

The separate staining with alizarin red for bones is the most commonly used in the routine teratological testing, since it involves a much easier and cheap procedure [1, 5]. However, this turns the staining of the cartilaginous part of the skeleton is impossible

and causes a failure in the identification of toxicologically important changes in the skeletal morphology [7].

The Wilson section method, which is standard teratological method and still represents the most utilized technique to examine the visceral organs, was used to explore the soft tissues [9].

Material and Methods

Experimental model for evaluation of the teratogenicity in rats

The study of teratogenicity required application of the studied substances on pregnant female Wistar rats (n=30) during the organogenesis (day 7-17) according to the International Conference on Harmonisation [4] (Fig. 1).

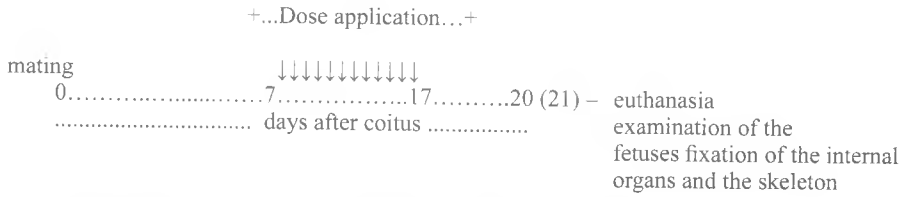

Fig. 1. Teratologic studies in rats (according to EEC, FDA, OECD, etc.).

On day 21 after conception were extracted via caesarian section and were explored for skeletal toxicity, using the method of "Double skeleton staining" with Alcian blue and Alizarin red [8].

The cartilages were stained in blue, and the ossified bones – in red (Fig. 2).

Fig. 2. Double skeleton staining method

The fetuses were studied microscopically to detect any internal organ (lung, liver, spleen etc.) abnormalities, using 20 body slices with thickness 1 mm. (Wilson Section Method) (Fig. 3).

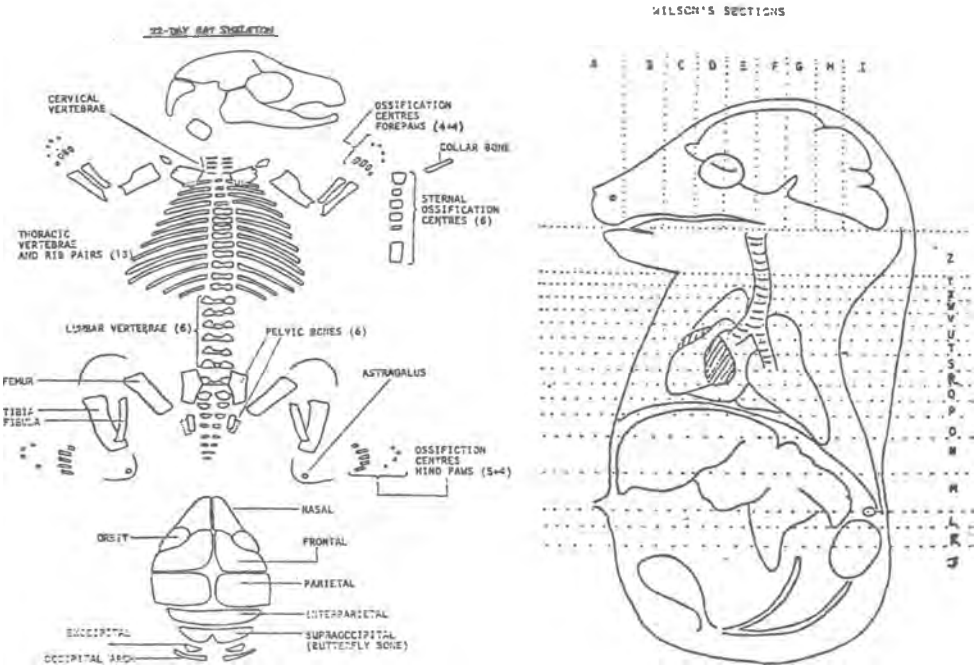

Fig. 3. Wilson Section Method

Results

The body weight and the length of the fetuses, the weight of the placentas and livers of adult rats treated with physiological solution are shown on Table 1.

On inspection of the fetuses from the group with physiological solution no anomalies were seen regarding the skeleton: head, body, extremities. Lack of teratogenic effect was detected, using following indices:

– Gross appearance – external;
– Coloration of the fetus;
– Subcutaneous hemorrhages.

Table 1. Weight and length of fetuses, weight of placentas and livers

	Weight fetuses (g)	Length fetuses (mm)	Weight placentas (g)	Weight livers (g)	n
Controls (phys. sol.)	2.81±0.24	33.48±1.21	0.45±0.007	0.20±0.017	29

Gross abnormalities as: spina bifida, anencephaly, exencephaly, arhinencephaly; cebocephaly;

Head: eyes, ears, nostrils, tongue, palate, mouth.

Extremities: anterior, posterior (number of fingers, syndactyly, micromelia).

Rear part of the body: anus (abnormalities) – atresia ani; tail (deformities, lack of tail; genitals (2 mm for male gender, 1 mm for female gender).

Test for skeletal toxicity

When the physiological solution was tested for skeletal toxicity with the "double staining method" all cartilages stained in blue, and the ossified bones – in red. The examination of the whole skeleton for variations in the level of ossification, lack of cartilages and any possible abnormalities no deviations from the norm were found. For each bone the dimensions, type, relative position, number of bones and ribs were denoted and compared to standarts. The physiological solution that was tested did not induce skeletal toxicity.

Microscopy

On microscopy examination of the fetuses for abnormalities of the internal organs (lung, liver, spleen, kidneys etc.) using the Wilson section method no anomalies were found in any of the explored organs.

Discussion

In the current experiment a test for fetal and maternal toxicity of the physiological solution was done. The body weight, and the length of the fetuses, the weight of the placentas, and livers of rats treated with physiological solution, did not show statistically significant difference compared to normal parameters. The inspection of the fetuses showed no abnormalities.

The physiological solution was tested for skeletal toxicity using double skeleton staining method with alizarin red and alcian blue [8]. All cartilages stained in blue, and the ossified bones in red. Thus the cartilaginous and the bone part of the skeleton were examined, and any possible abnormalities could be detected. The animals treated with physiological solution showed no difference compared to the norm.

The Wilson section method, which is standard teratological method and still represents the most utilized technique to examine the visceral organs, was used to explore the soft tissues [9]. No impairment was found in any of the examined internal organs.

This experiment convincingly demonstrates that physiological solution does not manifest data indicative for teratogenicity.

Conclusion

The double staining method used in the current study is a fundamental part of the teratological studies for assessment of the toxicity of the xenobiotics and non-chemical factors for the individual development. The double staining method described in the study is used by world-wide known toxicological laboratories, by universities and pharmaceutical corporations – Bayer Corporation, Lilly RL, Zeneca, Glaxo, Sanofi, etc. [10, 11, 12].

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